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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUN 23 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

SUBJECT: Review of Chronic Inhalation Study in
Rats with Methyl Bromide CAS No. 555

TO: Mr. Walter Francis
Disinfectants Branch
Registration Division (TS-767)

FROM: William L. Burnam *William L. Burnam/lem*
Deputy Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

This study was sent to me by Dr. Amy Rispin in Feb. 1987. As far as I know, it has not be formally submitted by the Methyl Bromide registrant in an attempt to fill a guideline requirement. It was performed in the Netherlands at the request of the Dutch government.

The study was carried out by the inhalation route at doses high enough to cause overt toxicity. No oncogenic effects were noted at the highest dose tested, however no individual animal data were submitted with this report so we could not verify the summary tables. The chronic phase of the study also suffers from lack of detailed data but, in addition, various clinical chemistry measurements (suggested by our guidelines) were not carried out. Other problems are mentioned in the Dynamac review. As the study exists now, it is supplementary for both chronic and oncogenic effects.

cc:
Amy Rispin

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CONFIDENTIAL
NATIONAL SECURITY INFORMATION (EO 12958)

EPA: 68-02-4225
DYNAMAC No. 362-A
May 31, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Chronic Inhalation Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: _____

Date: _____

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EPA: 68-02-4225
DYNAMAC No. 362-A
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DATA EVALUATION RECORD
METHYL BROMIDE
Chronic Inhalation Study in Rats

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DATA EVALUATION REPORT

TOX. CHEM. No.:
MRID No.:

STUDY TYPE: Chronic inhalation study in rats.

ACCESSION NUMBER: N/A.

TEST MATERIAL: Methyl bromide.

SYNONYM: N/A.

STUDY NUMBER: V86.22104.

SPONSOR: TNO Netherlands.

TESTING FACILITY: TNO Netherlands.

TITLE OF REPORT: Chronic (29-Month) Inhalation Toxicity and
Carcinogenicity Study of Methyl Bromide in Rats.

AUTHOR(S): Reuzel, P. G. J., Kuper, C. F., Dreef-van der Meulen, H. C.,
and Hollanders, V. M. H.

REPORT ISSUED: January 1987.

CONCLUSIONS:

In a chronic inhalation study in which Wistar rats were exposed to methyl bromide at levels of 0, 3, 30, or 90 ppm, compound-related increases in mortality at the high-dose level were noted. Deaths were associated with compound-related gross and histological heart effects, which included hemothorax, myocardial degeneration, and thrombi. Consistent, significant ($p < 0.05$) reductions in body weights and brain weights were also observed in the high-dose group when compared to the control. Inhalation of methyl bromide resulted in mild irritation of the nasal epithelia at all dose levels. Mild irritation of the esophagus and stomach as a consequence of ingesting the test material was also noted in the high-dose group. No compound-related neoplasms were observed; therefore, methyl bromide was not considered to be carcinogenic in rats. The NOEL and LOEL were 30 and 90 ppm, respectively, based on body weight and brain weight reductions and increased mortality with associated heart effects at 90 ppm.

Core Classification: Core Supplementary. This classification may be upgraded upon receipt of individual animal data from the sponsor.

A. MATERIALS:

1. Test Compound: Methyl bromide; description: colorless liquified gas; batch: none specified; purity: >98.8%; impurities: [REDACTED]
2. Test Animals: Species: rat; strain: Wistar (Cpb:Wu); age: six weeks old; weight: mean group body weights at initiation were 99-101 and 91-93 g for males and females, respectively; source: TNO Central Institute for Breeding of Laboratory Animals.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups:

Test Group	Exposure Level (ppm)	Main Study ^a		Satellite Groups (Interim ^b sacrifice)	
		Males	Females	Males	Females
1 Control	0	50	50	40	40
2 Low (LDT)	3	50	50	40	40
3 Mid (MDT)	30	50	50	40	40
4 High (HDT)	90	50	50	40	40

^a Males were exposed for 127 weeks and females were exposed for 129 weeks.

^b There were four satellite groups (a, b, c, d) with each containing 10 animals/sex. Groups of animals were killed after 13, 53, and 105 weeks on study. At weeks 13 and 53, satellite groups a and b were used for blood samples. Groups b and c were used for organ weights at weeks 53 and 105. Males from d were used for behavioral studies at week 41, and females from d were sacrificed at study termination (week 129).

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The exposure levels chosen were based on the results of two preliminary inhalation toxicity studies in which rats were exposed to methyl bromide for 6 hours/day, 5 days/week, for 13 weeks via inhalation. In the subacute study, exposure to 150 ppm resulted in high mortality and severe neurological effects (van Logten et al., 1980).¹ In the subchronic study, exposure levels of 1 to 42 ppm resulted in marginal changes in the liver (Wilmer et al., 1983).²

2. Exposure Chambers and Test Atmosphere Generation: The exposure chambers were cylindrical stainless-steel inhalation chambers with a 2.5-m³ capacity. The total air flow ranged from approximately 40 to 60 m³/hour. Temperature and relative humidity within the chambers were $21 \pm 2^\circ\text{C}$ and 45-70 percent, respectively. Animals were group housed (5/cage) in wire-mesh stainless-steel cages within the inhalation chambers during the entire length of the study.

Exposures were for 6 hours/day, 5 days/week, during the study. Generation of the test atmosphere was computer controlled through mass-flow controllers into a mixing device where the test material was diluted with filtered air from the air-conditioning system. Analysis of test material concentrations was performed using gas chromatography (GC). Triplet samples were collected and analyzed every half hour. The GC was connected with a microcomputer-interfaced serial data acquisition system in conjunction with a second microcomputer that monitored and controlled the exposure levels.

3. Food and Water Availability: Animals received food and water ad libitum during the nonexposure period. No food or water was available during the exposure period.
4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weights were analyzed using analysis of covariance and the Dunnett test. Mortality and pathology data were analyzed using the Fisher exact test. Organ weights and hematology and clinical chemistry data were analyzed using analysis of variance and the Dunnett test.
5. Quality Assurance: A quality assurance statement was signed and dated January 13, 1987.

C. METHODS AND RESULTS:

1. Concentration analyses: Mean chamber concentrations for the study were 3.7 ± 0.4 , 29.6 ± 1.9 , and 89.1 ± 3.4 ppm for the 3-, 30-, and 90-ppm exposure levels, respectively.

¹ van Logten, M. J., van der Heijden, C. A., and van Esch, G. J. (1980) RIVM report No. 149/80 CBS VI.

² Wilmer, J. W. G. M., Reuzel, P. G. J., and Dreef van der Meulen, H. C. (1983) CIVO report No. V82.378.

2. Observations: Animals were inspected twice a day during the week and once a day on weekends for signs of toxicity and mortality.

Results: There were no clinical signs of toxicity observed during the entire study. Increased mortality was observed in the high-dose group when compared to controls, particularly later in the study (Table 1). Significant increases ($p < 0.05$) in cumulative mortality occurred at weeks 114 and 118 in high-dose males and at weeks 121 and 125 in high-dose females when compared to control values. Mortality was comparable between controls and low- and mid-dose groups throughout the study.

3. Body Weight: Rats were weighed once weekly for 3 months, then once every 4 weeks for the remainder of the study.

Results: The body weights of high-dose males and females were consistently lower than those for control animals throughout the study (Table 2). Significant decreases ($p < 0.05$) in body weight were noted frequently in high-dose males and continuously in high-dose females from week 4 on. Body weights of the low- and mid-dose groups were comparable to controls, although significant decreases ($p < 0.05$) were occasionally noted.

4. Food Consumption: Consumption was not determined.

5. Ophthalmology: Ophthalmological examinations were not performed.

6. Hematology and Clinical Chemistry: Blood was collected from the tip of the tail at 13 and 52 weeks for hematology and glucose analyses from 10 rats/sex/group. Blood samples for clinical chemistry analysis were obtained from the abdominal aorta of these animals under ether anesthesia. Baseline values were apparently not measured prior to exposure. The CHECKED (X) parameters were examined:

a. Hematology

X Hematocrit (HCT) [†]	X Leukocyte differential count
X Hemoglobin (HGB) [†]	Mean corpuscular HGB (MCH)
X Leukocyte count (WBC) [†]	Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC) [†]	Mean corpuscular volume (MCV)
Platelet count [†]	Coagulation:thromboplastin time (PT)
Reticulocyte count (RETIC)	Total plasma protein (TP)
Red cell morphology	

Results: At the 13-week interval, hematocrit values for high-dose females were significantly decreased ($p < 0.05$) when compared to controls. In addition, there were significant increases ($p < 0.05$) in neutrophil count in low-dose females and significant decreases in erythrocyte counts in mid-dose males when compared with control values.

[†]Recommended by Subdivision F (October 1982) Guidelines.

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TABLE 1. Effects of Lifetime Inhalation Exposure to Methyl Bromide on Cumulative Mortality in Rats

Exposure Level (ppm)	Cumulative Mortality (percent survival) at Week:				
	55	78	107	118	128
<u>Males</u>					
0	1(98)	1(98)	16(68)	26(48)	35(30)
3	0(100)	2(96)	13(74)	20(60)	25(50)
30	0(100)	2(96)	17(66)	24(52)	34(32)
90	1(98)	4(92)	26(48)	38(24)*	42(16)

<u>Females</u>					
0	0(100)	4(92)	16(68)	23(54)	35(30)
3	1(98)	4(92)	15(70)	19(62)	27(46)
30	1(98)	5(90)	19(62)	29(42)	33(34)
90	1(98)	3(94)	23(54)	32(36)	43(14)

*Significantly different from control value at $p < 0.05$.

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TABLE 2. The Effect of Inhalation of Methyl Bromide on Mean Body Weights (g) in Rats After 2 Years of Exposure^a

Exposure Level (ppm)	Mean Body Weight (\pm S.D.) at Study Week:						Termination
	0	12	28	52	76	104	
<u>Males</u>							
0	100 \pm 0.9	360 \pm 3.7	428 \pm 4.1	488 \pm 5.0	508 \pm 5.8	516 \pm 8.4	454 \pm 13.2
3	101 \pm 1.0	367 \pm 3.8	426 \pm 4.8	491 \pm 6.7	505 \pm 8.8	514 \pm 11.3	449 \pm 10.0
30	99 \pm 1.0	366 \pm 3.8	429 \pm 4.6	488 \pm 5.9	512 \pm 6.8	522 \pm 8.8	418 \pm 14.9
90	101 \pm 1.0	345 \pm 3.7**	416 \pm 4.3	469 \pm 5.2*	490 \pm 6.6	498 \pm 11.2	431 \pm 23.3

<u>Females</u>							
0	92 \pm 0.7	219 \pm 1.7	254 \pm 2.5	300 \pm 3.8	328 \pm 4.8	347 \pm 5.6	304 \pm 11.9
3	93 \pm 0.7	218 \pm 1.8	250 \pm 2.4	292 \pm 3.5	321 \pm 4.9	338 \pm 5.8	276 \pm 7.7*
30	92 \pm 0.9	220 \pm 1.9	249 \pm 2.4	293 \pm 3.7	327 \pm 5.0	341 \pm 7.6	286 \pm 8.7
90	91 \pm 0.8	212 \pm 1.8**	242 \pm 2.5**	284 \pm 3.9**	306 \pm 4.9**	324 \pm 7.3	267 \pm 10.2*

^aBody weights were recorded on week 125 for males and week 128 for females.*Significantly different from control value at $p < 0.05$.**Significantly different from control value at $p < 0.01$.

At week 52, white blood cell counts of low- and high-dose males were significantly increased ($p < 0.05$) when compared to control values. No other differences in hematological indices were noted.

b. Clinical Chemistry

<u>Electrolytes</u>		<u>Other</u>	
Calcium [†]		X	Albumin [†]
Chloride [†]			Albumin/globulin ratio
Magnesium [†]			Blood creatinine [†]
Phosphorus [†]		X	Blood urea nitrogen [†]
Potassium [†]			Cholesterol [†]
Sodium [†]			Globulins
		X	Glucose [†]
<u>Enzymes</u>			Total bilirubin [†]
X	Alkaline phosphatase (ALP)	X	Total protein [†]
	Cholinesterase		Triglycerides
	Creatinine phosphokinase [†]		Direct bilirubin
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT) [†]		
X	Serum aspartate aminotransferase (SGOT) [†]		
	Gamma glutamyltransferase (GGT)		
	Urea		

Results: Alkaline phosphatase values were significantly increased ($p < 0.05$) for mid-dose females when compared to controls at the 13-week interval. However, values for males at week 13 and males and females at week 53 from the methyl bromide-exposed groups were comparable to control values.

At week 53, there were dose-related decreases (significant at the mid- and high-dose levels, $p < 0.05$) in blood urea values for methyl bromide-exposed males when compared to controls (Table 3). These changes were not noted in females, however. Small, but significant, increases ($p < 0.05$) in total protein values were observed in high-dose males but not in females. Significant decreases in serum alanine aminotransferase ($p < 0.01$) and serum aspartate aminotransferase ($p < 0.05$) were noted in high-dose females when compared to controls. These enzyme levels were comparable between methyl bromide-exposed males and controls.

7. Urinalysis: Urine was collected overnight from fasted animals at 13 and 53 weeks. The CHECKED (X) parameters were examined:

X	Appearance [†]	X	Glucose [†]
X	Volume [†]	X	Ketones [†]
X	Specific gravity [†]		Bilirubin [†]
X	pH	X	Blood [†]
X	Sediment (microscopic) [†]		Nitrate
X	Protein [†]		Urobilinogen

[†]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. The Summary of Effects of Methyl Bromide Exposure on Clinical Chemistry Parameters in Rats^a

Clinical Chemistry Parameter	Mean Values (\pm S.D.) at Exposure Level (ppm):									
	Males					Females				
	0	3	30	90		0	3	30	90	
Urea (mmol/L)	6.37 \pm 0.23	5.92 \pm 0.48	5.40 \pm 0.18*	5.30 \pm 0.17*	(10) ^b	6.11 \pm 0.35	6.29 \pm 0.23	6.16 \pm 0.38	6.06 \pm 0.22	(10)
Serum alanine transferase (u/L)	49.2 \pm 3.8	44.8 \pm 2.8	44.4 \pm 3.3	40.9 \pm 3.8	(9)	58.1 \pm 8.2	44.4 \pm 2.9	42.1 \pm 2.6	35.6 \pm 1.7**	(8)
Serum aspartate aminotransferase (u/L)	66.1 \pm 3.5	66.0 \pm 2.6	65.3 \pm 3.8	66.6 \pm 4.4	(10)	94.0 \pm 13.6	69.9 \pm 4.5	75.0 \pm 4.1	64.3 \pm 3.2*	(10)

^a Includes only animals sacrificed at week 53.

^b Number in parentheses are the number of animals sampled.

* Significantly different from control value at $p < 0.05$.

** Significantly different from control value at $p < 0.01$.

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Results: There were no changes in urinary indices that suggested an adverse effect associated with inhaling methyl bromide.

8. Sacrifice and Pathology: All animals that died or that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected and preserved in a 4 percent buffered formalin solution for histological examination. In addition, the (XX) organs from animals killed at 53 and 104 weeks were weighed:

<u>Digestive system</u>		<u>Cardiovasc./Hemat.</u>	<u>Neurologic</u>
	Tongue	X Aorta†	XX Brain†
X	Salivary glands†	XX Heart†	X Peripheral nerve
	(sublingual, sub-maxillary, parotid)	X Bone marrow†	(sciatic nerve)†
X	Esophagus†	(sternum)	X Spinal cord (3 levels)
X	Stomach†	X Lymph nodes†	XX Pituitary†
X	Duodenum†	(mediastinal, mesenteric)	X Eyes (optic nerve)†
X	Jejunum†	XX Spleen†	
X	Ileum†	XX Thymus†	<u>Glandular</u>
X	Cecum†		XX Adrenals†
X	Colon†	<u>Urogenital</u>	X Harderian glands
	Rectum†	XX Kidneys†	X Lacrimal gland
XX	Liver†	X Urinary bladder†	X Mammary gland†
X	Pancreas†	XX Testes†	X Parathyroids†
		X Epididymides	X Preputial/clitoral glands
		X Prostate	X Thyroids†
	<u>Respiratory</u>	X Seminal vesicle	X Zymal glands
X	Trachea†	XX Ovaries	
X	Larynx	X Uterus†	<u>Other</u>
XX	Lung†		X Bone (sternum)†
X	Nasal cavity		X Skeletal muscle†
X	Pharynx		X Skin
			X All gross lesions and masses

The lungs were inflated with fixative under 10-cm water pressure and the nasal cavity was sectioned at four levels and examined.

Histologic examination at weeks 53 and 105 (interim sacrifices) was performed on all tissues and organs from control and high-dose rats. In addition, the nasal cavity (target organ) of low- and mid-dose animals was examined.

All tissues, organs, and gross lesions from animals that died or were sacrificed moribund during the study and control and high-dose animals surviving to terminal sacrifice (week 127 for males and week 129 for females) as well as female rats from satellite group d were histologically examined. In addition, the adrenals, brain, heart, liver, nasal cavity, esophagus, ovaries/testes, pituitary, stomach, uterus, thyroid, and gross lesions in low- and mid-dose rats from the main study and females from satellite group d were examined.

†Recommended by Subdivision F (October 1982) Guidelines.

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Results:

- a. Organ Weights: There were significant decreases ($p < 0.05$) in absolute kidney weights for high-dose males and mid- and high-dose females at the 53-week interval (Table 4). Relative (organ-to-body weight) kidney weights were significantly decreased ($p < 0.05$) for mid- and high-dose males at week 53 when compared to controls. The decreases were dose related for both males and females. Absolute brain weights were significantly decreased ($p < 0.05$) for high-dose females at both week 53 and 104 when compared to controls. Similarly, absolute brain weights for high-dose males were slightly, but not significantly, lower than controls.

However, relative brain weights for animals exposed to methyl bromide were comparable to control values. No other statistically significant changes in absolute or relative organ weights were noted.

- b. Gross Pathology: There were no gross lesions or other abnormalities found at the 53- or 105-week interim necropsies that could be attributed to the exposure of methyl bromide.

The incidence of hemothorax was increased in high-dose animals found dead or killed in extremis when compared to controls. There was an increased incidence of discolored lungs in high-dose females when compared to controls. No other adverse effects were noted. However, there were significantly decreased ($p < 0.05$) incidences of females with mammary glands exhibiting secretory activity and possible mammary tumors and males with discolored kidneys in the high-dose group when compared to controls.

- c. Microscopic Pathology:

- 1) Nonneoplastic: The authors reported a significant dose-related trend (p value for trend not reported) towards an increased incidence and severity of nasal effects in rats exposed to methyl bromide when compared to controls (Table 5). These effects were restricted to the dorso-media area of the nasal cavity and were characterized by basal cell hyperplasia, occasionally accompanied by cystic structures, and degeneration of the epithelial layer. Occasionally noted were clusters of sensory-like cells in the hyperplastic basal cell layer. The authors reported that these changes were first noted at week 53. However, there was no progression of severity as the exposure period increased. The remainder of the respiratory tract was unaffected by exposure to methyl bromide.

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TABLE 4. The Effects of Chronic Inhalation of Methyl Bromide on Organ Weights (mean \pm S.D.) of Rats^a

Exposure Level (ppm)	Brain		Kidney		Liver	
	g	g/kg	g	g/kg	g	g/kg
<u>Males</u>						
0	2.01 \pm 0.03	4.40 \pm 0.14	2.71 \pm 0.11	5.91 \pm 0.25	14.03 \pm 0.64	30.5 \pm 1.1
3	2.12 \pm 0.01*	4.25 \pm 0.13	2.73 \pm 0.09	5.43 \pm 0.06	14.75 \pm 0.88	29.3 \pm 1.2
30	2.03 \pm 0.03	4.20 \pm 0.15	2.58 \pm 0.06	5.33 \pm 0.16*	14.29 \pm 0.87	29.1 \pm 1.0
90	1.93 \pm 0.03	4.30 \pm 0.14	2.33 \pm 0.08*	5.13 \pm 0.08**	12.62 \pm 0.49	27.8 \pm 0.5
<u>Females</u>						
0	1.94 \pm 0.02	6.79 \pm 0.30	1.88 \pm 0.06	6.54 \pm 0.21	8.50 \pm 0.47	29.1 \pm 0.5
3	1.95 \pm 0.03	6.59 \pm 0.18	1.83 \pm 0.07	6.16 \pm 0.22	9.13 \pm 0.31	30.7 \pm 0.7
30	1.85 \pm 0.04	6.62 \pm 0.26	1.66 \pm 0.04*	5.96 \pm 0.24	8.03 \pm 0.36	28.5 \pm 1.0
90	1.81 \pm 0.03**	6.66 \pm 0.28	1.64 \pm 0.06*	5.99 \pm 0.15	8.07 \pm 0.45	29.3 \pm 1.0

^aData presented were collected at week 53.*Significantly different from control value at $p < 0.05$.**Significantly different from control value at $p < 0.01$.

TABLE 5. Histologic Findings in the Nasal Cavity of Rats Exposed to Methyl Bromide

Lesion	Exposure Level (ppm)							
	Males				Females			
	0	3	30	90	0	3	30	90
<u>Interim Sacrifice 1 (370-371 days)</u>								
	(10) ^a	(8)	(9)	(10)	(10)	(8)	(8)	(10)
Basal cell hyperplasia								
-very slight to slight	3	0	1	2	0	0	1	2
-moderate	0	0	0	3	0	0	0	0
<u>Interim Sacrifice 2 (734-735 days)</u>								
	(10)	(7)	(9)	(9)	(9)	(10)	(10)	(8)
Basal cell hyperplasia								
-very slight to slight	3	0	4	4	3	2	4	4
-moderate	0	0	0	2	1	0	1	3
<u>Main Study and Satellite Group d</u>								
	(46)	(48)	(49)	(48)	(58)	(58)	(59)	(59)
Basal cell hyperplasia								
-very slight to slight	4	12	19	22	9	19	22	33
-moderate	0	1	4	9**	0	0	3	9**
-total	4	13*	23**	31**	9	19*	25*	42**
Hyperkeratosis	0	0	0	3	1	0	0	2
Epithelial hyperplasia	0	4	1	2	6	3	1	9

^aThe number of animals with tissue examined histologically is in parentheses.

* Significantly different from control value at $p < 0.05$.

** Significantly different from control value at $p < 0.01$.

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There were several histological changes noted in the heart that suggested a compound-related effect. Slight increases (not significant) in the incidences of thrombi, and cartilaginous metaplasia were noted in high-dose rats during the 105-day interim sacrifice. Also observed were slight increases in the incidence of moderate to severe myocarditis in high-dose males. There was an increased incidence (significant at $p < 0.01$) of heart thrombi in high-dose animals surviving more than 2 years when compared to controls (Table 6). The authors reported that thrombi were noted mostly in animals found dead or killed moribund during the study. The incidence of cartilaginous metaplasia of the heart was significantly increased ($p < 0.05$) in high-dose males and low-dose females when compared to controls. There was also a significant increase ($p < 0.01$) in the incidence of moderate to severe myocardial degeneration in high-dose females and a nonsignificant increase in high-dose males.

Several changes found in the digestive tract were also attributed to the inhalation of methyl bromide. A higher incidence of hyperkeratosis of the esophagus was noted in high-dose animals when compared to controls. This increase was significant ($p < 0.01$) in high-dose males. There was also a higher degree of severity associated with the esophageal hyperkeratosis noted at the high-dose level. A slight, nonsignificant increase in the incidence of hyperkeratosis in the stomach was also noted in the high-dose group. The incidence of bile duct proliferation was significantly higher ($p < 0.01$) in high-dose females than controls, but incidences were comparable between control and high-dose males. Table 7 summarizes the types of nonneoplastic lesions observed in this study.

- 2) Neoplastic: There were no increases in the incidence of any neoplastic lesions that were attributable to methyl bromide exposure (Table 8). Conversely, there were significant decreases ($p < 0.05$) in the incidences of fibroadenomas of the mammary gland in high-dose females and of pheochromocytomas of the adrenals in high-dose males when compared to controls.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded that chronic exposure to methyl bromide via inhalation resulted in decreases in body weight and brain weight and increases in mortality that were associated with increased incidences of hemothorax, myocardial degeneration, and thrombi of the heart in rats at an exposure level of 90 ppm.

Increased incidences of hyperkeratosis in the esophagus and the forestomach of high-dose animals were considered by the authors to be due to irritation following the ingestion of seromucous fluids from the respiratory tract.

TABLE 6. Summary of Heart Effects Found in Rats Exposed to Methyl Bromide^a

Lesion	Number (% Incidence) of Heart Lesions at Exposure Level (ppm):							
	Males				Females			
	0	3	30	90	0	3	30	90
Thrombus	5(10)	3(6)	10(20)	21(43)*	5(8)	11(9)	3(5)	20(33)**
Cartilaginous metaplasia	2(4)	4(8)	7(14)	12(24)**	6(10)	18(31)*	3(5)	14(23)
Myocardial degeneration								
-very slight to slight	16(33)	19(38)	29(58)	8(16)	33(56)	31(53)	40(68)	13(22)
-moderate to severe	31(65)	28(56)	16(32)	41(84)	24(41)	22(37)	16(27)	44(73)**

^a Includes only animals in the main study.

* Significantly different from control value at $p < 0.05$.

** Significantly different from control value at $p < 0.01$.

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TABLE 7. Selected Nonneoplastic Lesions in Rats Exposed to Methyl Bromide for 29 Months^a

Organ/Lesion	Exposure level (ppm)							
	Males				Females			
	0	3	30	90	0	3	30	90
<u>Esophagus</u>	(46) ^b	(48)	(49)	(48)	(58)	(58)	(59)	(59)
Hyperkeratosis								
Very slight to slight	17	9	24	24	17	16	20	21
Moderate to severe	1	3	5	8	3	3	5	10
Total	18	12	29	32**	20	19	25	31
<u>Stomach</u>	(46)	(49)	(49)	(46)	(60)	(60)	(59)	(59)
Hyperkeratosis	14	11	18	24	15	17	20	21
Papillomatosis	1	0	2	4	3	1	2	2
<u>Liver</u>	(48)	(48)	(49)	(49)	(60)	(60)	(59)	(60)
Focus of cellular alteration	0	0	4	0	1	2	5	0
Hyperplastic nodule(s)	0	2	1	1	0	1	1	0
Bile duct proliferation	6	5	6	8	5	13	6	17*
<u>Pituitary</u>	(47)	(50)	(50)	(44)	(58)	(58)	(58)	(54)
Hyperplastic focus	5	6	7	5	4	6	10	10
<u>Thyroid</u>	(46)	(49)	(46)	(46)	(55)	(58)	(57)	(59)
Parafollicular cell proliferation	5	17*	5	6	6	15	11	11
<u>Uterus</u>					(58)	(60)	(59)	(58)
Endometrial hyperplasia					3	5	4	6

^a Includes only animals in the main study.^b The number in parentheses is the number of tissues examined.* Significantly different from control value at $p < 0.05$.** Significantly different from control value at $p < 0.01$.

A.

TABLE 8. Representative Neoplastic Lesions in Rats Exposed to Methyl Bromide^a

Organ/Lesion	Exposure level (ppm)							
	Males				Females			
	0	3	30	90	0	3	30	90
<u>Adrenals</u>	(48) ^b	(50)	(48)	(49)	(59)	(60)	(59)	(60)
Pheochromocytoma	10	11	9	2*	2	2	0	1
<u>Brain</u>	(47)	(50)	(50)	(49)	(60)	(58)	(58)	(60)
Glioma	0	0	3	0	0	0	1	0
<u>Mammary gland</u>					(60)	(27)	(35)	(59)
Fibroadenoma					29	26	32	13**
<u>Nasal cavity</u>	(46)	(9)	(1)	(48)	(58)	—	—	(59)
Squamous cell carcinoma	1	2	0	0	0			0
<u>Pituitary</u>	(47)	(50)	(50)	(44)	(58)	(58)	(58)	(54)
Hemorrhagic tumor	10	21*	14	7	24	22	27	17
<u>Testes</u>	(46)	(50)	(48)	(47)				
Leydig cell tumor	0	4	2	1				
<u>Uterus</u>					(58)	(60)	(59)	(58)
Fibromatous polyp					10	10	7	7

^a Includes only animals in the main study and females from satellite group d.^b The number in parentheses is the numbers of tissues examined.* Significantly different from control value at $p < 0.05$.** Significantly different from control value at $p < 0.01$.

Brain weights were significantly lower than controls at the high-dose level. However, there were no histological changes or clinical observations during the study to indicate neurological effects. In addition, the results of a neurotoxicological study performed at the same time did not reveal any neurotoxic effects.

The significant decreases in serum urea and reduced kidney weights at week 53 were considered to be unrelated to methyl bromide exposure because there were no histological changes noted in the kidneys and similar effects were not observed in rats at 104 weeks.

The results of this study also indicated that methyl bromide is a mild irritant to the nasal epithelia of rats at all exposure levels tested. Methyl bromide was not carcinogenic to rats at the exposure levels tested.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

We agree with the study authors' assessment on the chronic toxicity of methyl bromide, and the rationale for dose selection appears adequate. There was an increase in mortality in the high-dose group towards the end of the study. Histological evidence suggests that the shortened lifespan noted in the high-dose animals was due to compound-related heart effects, which included increased incidences of thrombi, myocardial degeneration, and cartilaginous metaplasia. Gross evidence of pulmonary toxicity (hemothorax) was also observed.

Mild irritation of the nasal epithelia in rats exposed to methyl bromide was not ~~d~~. There was also compound-related irritation in the upper digestive tract and stomach. This was probably due to the ingestion of methyl bromide from grooming behavior as well as swallowing of nasal secretions.

There were several deficiencies in the conduct and reporting of this study, some of which may have impaired the authors' assessment of the results. These are as follows:

1. No individual animal data were presented in this report. Therefore, summary data could not be verified.
2. The clinical chemistry analyses were not adequate according to the U.S. EPA guidelines. Clinical chemistry parameters not analyzed were electrolytes, creatinine phosphokinase, blood creatinine, cholesterol, and total bilirubin. In addition, clinical chemistry and hematology analyses and urinalysis were performed at 13 and 53 weeks only. No analyses were performed prior to the study initiation, at 104 weeks, or at study termination.

More complete clinical chemistry data would have been helpful to assess some of the results obtained in this study. For example, no electrolytes were assayed. There were apparent kidney effects noted during the study, which may have been better evaluated using electrolyte and creatinine assay results. The heart effects noted could have been further clarified by assaying lactic dehydrogenase and electrolytes.

3. No ophthalmologic examinations were performed; therefore, possible eye effects were not evaluated. This exam would probably have yielded interesting results considering the amount of light that the animals were exposed to daily. The authors reported that the chambers were illuminated from an external source and each cage within the chamber cage received approximately 1 to 7 lumen (1 to 7 foot candles) of light from the single window of the chamber. Hayes recommends that animals receive 75 to 125 foot candles of light³. Therefore, the rats in this study were deprived of sufficient light during the study and probably had serious vision problems by study termination. The eyes were examined grossly and histologically and no abnormalities were reported.
4. Food consumption was not measured and, therefore, changes in body weights could not be compared to or verified by decreases in food consumption.

³ Hayes, A. W. ed. (1982). Principles and Methods of Toxicity. Raven Press, New York, p. 328.

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